Activation of the Murine Sarcoma Virus Genome After Infection with the Murine Leukemia Virus as Determined by Cell Agglutination

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Non-virus-producing NIH/3T3 cells transformed by the murine sarcoma virus are agglutinated by conconavalin A to the same low level as normal NIH/3T3 cells. Infection with the murine leukemia virus greatly increases the agglutination of transformed cells but not that of normal cells. These data suggest that the morphological expression of cell transformation and the surface alterations associated with increased cell agglutination are controlled by the expressions of different sarcoma virus genes.

Agglutination of cells by plant agglutinins (lectins) has been used to monitor surface changes in transformed cells (5, 10, 17). A correlation between the degree of agglutinability and the malignancy of cells transformed by DNA tumor viruses and by chemical carcinogens was reported recently (9). Cells transformed and continuously producing the sarcoma-leukemia viruses of avian, murine, and feline origin are also agglutinated by lectins such as wheat germ agglutinin and concanavalin A (con A) (4, 6, 11, 12, 14). However, cells producing the leukemia virus component only and non-virus-producing cells transformed by the sarcoma virus agglutinate to a much lower degree (14). These findings suggest that agglutination of cells transformed by RNA tumor viruses may not necessarily be associated with cell transformation but rather with the expression of specific sarcoma virus genetic elements.

To further explore the relationship between virus replication and cell agglutinability, we have studied several non-virus-producing (NP) cells transformed by the murine sarcoma virus (MSV). It has been shown that the sarcoma genome can be rescued from MSV-transformed NP cells by superinfection with the murine leukemia virus (MLV) (1, 2, 8) resulting in the release of a sarcomagenic virus that can transform normal murine cells. We report here that superinfection with MLV of MSV-transformed NP mouse cells results in a surface change that is revealed by increased agglutination by con A. In effect, MLV activates the expression of sarcoma genetic information that is not expressed before superinfection.

MATERIALS AND METHODS

Cells and virus. Four NP clonal lines derived from the same parental cell line of MSV-transformed murine NIH/3T3 fibroblasts were generously provided by George J. Todaro. The cells were maintained in Dulbecco modified Eagle medium (EM) and supplemented with 10% calf serum.

The Moloney strain of MLV was obtained from 2- to 3-day-old culture fluids of MLV-producing NIH/3T3 cells that were grown in Eagle minimal essential medium supplemented with 10% calf serum. The culture fluids were harvested in the cold, centrifuged for 15 min at 1,500 × g, and used directly as a stock of virus. The titer as determined by XC-test (13) was 5.8 × 10^3 PFU/cell.

Determination of agglutinability. Cells were tested for agglutinability as previously described (15).

RESULTS

Agglutinability of NP clonal lines. The cell density and degree of agglutination by con A of four NP clonal lines were determined at daily intervals after seeding. As shown in Fig. 1A, 71N cl.6 was agglutinated 12% at 500 μg of con A per ml, similar to that of normal NIH/3T3 fibroblasts. Clones 71Ncl.2 and 71Ncl.5 agglutinated from 30 to 50% at 500 μg/ml. In contrast, the MSV(MLV)-producing transformed mouse cells agglutinated 70 to 90% under these conditions (14). No correlation was found between agglutinability and the degree of cell growth, because as shown in Fig. 1B, 71Ncl.6 cells that agglutinated the least grew to a saturation density similar to that of the other clones (Fig. 1B).

We next tested the agglutinability of 71Ncl.6 cells after infection with MLV (Moloney strain). As a control, NIH/3T3 cells were infected with...
MLV under identical conditions. An increase in agglutinability by con A of 71Ncl.6 cells was detected at day 2 after infection (Fig. 2A). The major increase occurred 3 to 4 days after infection when 60% of the cells were agglutinated by 250 μg of con A per ml. NIH/3T3 cells infected under identical conditions did not agglutinate more than uninfected cells (Fig. 2B). Agglutination in the presence of other concentrations of con A yielded similar kinetics (Table 1).

**Virus production by NP cells after infection with MLV.** The focus-forming ability of culture fluids from 71Ncl.6 and NIH/3T3 after infection with MLV is shown in Table 2. Sarcomagenic activity as measured by focus formation on NIH/3T3 was detected on day 2 after infection of 71Ncl.6 cells and increased on days 3 and 4. Culture fluids from uninfected 71Ncl.6 cells, and from infected or uninfected NIH/3T3 cells, did not form foci on NIH/3T3 cells (Table 2), or on BALB/3T3 cells (unpublished data). The release of leukemia virus, as determined by syncytia formation on XC-cells (13), could be detected by 24 h after infection of both 71Ncl.6 and NIH/3T3 cells.

**DISCUSSION**

Our results show that MSV-transformed, nonproducer cells are not necessarily agglutinated by con A, and that the rescue of the MSV genome by superinfection with MLV results in a markedly increased cell agglutination. These findings support earlier conclusions that surface alterations in MSV-transformed cells are controlled by the expression of sarcoma virus genes (14). Our results suggest that two
TABLE 1. Agglutinability of 71 cl.6 and NIH/3T3 cells after infection with MLV

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Days after infection</th>
<th>Con A (µg/ml)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NP</td>
</tr>
<tr>
<td>71 cl.6</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
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<tr>
<td></td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>NIH/3T3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>

* Cells were seeded, infected, and tested for agglutinability as described in the legend of Fig. 2.
* Agglutination %.
* NI, Not infected.
* I, Infected.

TABLE 2. Assay for MSV and MLV production by NIH/3T3 and 71 cl.6 cells after infection with MLV

<table>
<thead>
<tr>
<th>Days after infection</th>
<th>NIH/3T3 cells (FFU/ml)</th>
<th>71N-6 cells (FFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MSV</td>
<td>MLV</td>
</tr>
<tr>
<td>1</td>
<td>3.2 × 10^3</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>5.0 × 10^3</td>
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</tr>
<tr>
<td>3</td>
<td>2.1 × 10^4</td>
<td>7.0 × 10^3</td>
</tr>
<tr>
<td>4</td>
<td>7.0 × 10^4</td>
<td>5.2 × 10^3</td>
</tr>
<tr>
<td>5</td>
<td>2.1 × 10^4</td>
<td>7.0 × 10^3</td>
</tr>
</tbody>
</table>

* Cells were seeded and infected as described in the legend of Fig. 2. The medium was changed daily in all cultures. At the indicated times after infection, culture fluids were collected, clarified by centrifugation for 15 min at 1500 × g, and stored at -70 C. Focus assays for the determination of MSV production (16) and the XC-test for MLV production (13) were performed as described.
* FFU, Focus-forming units.
* PFU, Plaque-forming units as determined by the number of syncytia in the XC-test.

sets of MSV genes may function independently, one that controls the expression of the transformed cell morphology and the second that controls a cell surface alteration manifested by an increased agglutinability. Whether increased agglutinability results from a direct action of an MSV gene product, an interaction between MSV and MLV gene products, or a possible effect of MSV genes on cellular genes is not known. That MSV gene(s) associated with the morphological expression of transformation can also be rescued by MLV is suggested by the finding that an MSV-infected nonproducer 3T3 cell with a normal morphology can be transformed upon superinfection with MLV (2).

Our data illustrate that in vitro cell transformation is not necessarily associated with an increase in cell agglutinability by con A. It has previously been shown that cells productively infected with nononcogenic viruses, including myxoviruses and paramyxoviruses (3), vaccinia virus (18), and a nononcogenic adenovirus 12 mutant (15), also show an increased agglutinability. However, the nature of the surface changes in the case of productive infection with cytoidal viruses may be different than that induced by MSV genes.

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LITERATURE CITED